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FORM REGULATION IN CERIANTHUS, II.

The Effect of Position, Size and Other Factors upon Regeneration.—(*Continued.*)

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II. Discussion of Results.

The Factor of Position.

In the experiments described above, which were selected from a large number, the effect of position on regeneration has appeared in every case. Considering first the effect of position on the rapidity of regeneration, we find that in each series the rapidity of regeneration is dependent on the position which the piece occupied in the parent body, a decrease in the rapidity of regeneration occurring with increasing distance from the oral end. This relation holds for the aboral ends of pieces as well as for the oral ends, though the regenerative phenomena at the aboral ends are in most cases less sharply defined than at the oral ends. Further analysis is possible in such series as 54 and 55. The oral ends of the two sets of pieces 54*A* and 55*A* are situated at approximately the same level of the body, but the aboral ends of the pieces 54*A* are much nearer the aboral end of the parent body than those of 55*A*. The data given above for this series show very clearly that the rapidity of regeneration at the oral ends of both sets of pieces was equal, except in the final stages, while at the aboral ends it was much greater in the shorter pieces, *i. e.*, those in which the aboral cut surface was near the oral end of the parent body. From these experiments we may conclude that the two ends of a piece regenerate independently of each other, at least to a certain extent, the result depending in each case on the level which a particular cut surface occupied in the parent body.

Near the aboral end the regenerative capacity is reduced nearly or quite to zero, cut surfaces at this level showing no regeneration beyond the union of ectoderm and entoderm over the cut muscular layer.

The influence of temperature in determining the level at which the regenerative power is reduced to zero will be discussed in another section. As regards the amount of regeneration, the effect of position is similar to its effect on rapidity. Not only is there a decrease in the rapidity of regeneration toward the aboral ends, but the total amount of oral or aboral regeneration also decreases in the same manner. In certain cases, as in Series 54, 55 and 35, the difference in size of pieces may counteract the effect of position in later stages of regeneration, so that short pieces show slightly less oral regeneration than long pieces with oral ends at the same level (Series 54*A* and 55*A*), or the amount of oral regeneration on long aboral pieces may equal that on much shorter pieces from a region nearer the oral end (Series 35, *A* and *B*). But aside from exceptions of this kind the rapidity and the amount of regeneration depend upon the level in the parent body from which the cut surface is taken, both being greatest at the oral end and decreasing aborally.

The existence of a difference in regenerative power at different levels of the body being established, the question as to its nature and significance next requires consideration. I believe that it may be due in part to a difference in the general reactive capacity of the tissues in the different regions, *i. e.*, to a decrease in the reactive power of the tissues with increasing distance from the oral end. That such a difference does exist is indicated by various facts in the normal anatomy and physiology of the animal. We must consider first the anatomical features which bear upon this point. There is a marked decrease in the thickness of the body-wall and especially of the muscular layer, toward the aboral end; the number of mesenteries also decreases toward the aboral end, until only a single pair remains; all new mesenteries appear first at the oral end and extend gradually aborally, thus indicating that growth in circumference begins orally. The physiological differences of different regions which have been noted are: the greater sensitiveness of the oral region to tactile and other stimuli, and the greater contractility of the muscles of this region when stimulated.

Since I am convinced that the power of regeneration is due, not to any special regulatory mechanism, but to those properties of organized matter which cause "normal" or "typical" growth and differentiation, I believe that the differences in regenerative power and the differences in normal anatomical and physiological reactive capacity must all depend, at least in some degree, on the general reactive capacity of the tissues.

The differences in reactive capacity in different regions may be due to differences in the intensity of metabolic change, or they may depend upon other conditions. It is of interest to note in this connection that the differentiation and concentration of nervous tissue is greatest about the oral end in the actinians. It is at least not impossible that this fact is correlated in some manner with those above mentioned. For the present, however, the possibility of such a correlation is merely suggested as the data are still insufficient for positive conclusions.

There is also a possibility that the difference in the rapidity and amount of regeneration at different levels may be due in part to differences in internal pressure resulting from circulatory currents in the enteron. Discussion of this phase of the problem is, however, reserved for a future time.

The Factor of Size.

My observations along this line are to a large extent merely incidental, since, owing to the relatively slight influence of this factor, its effects were not clearly recognized until it was too late to complete the series of experiments necessary for further study. Certain conclusions may, however, be drawn from my work and these are briefly mentioned here.

It is evident from Series 54 and 55 that great differences in size of pieces do not affect the rapidity of regeneration in earlier stages. The pieces 54*A* and 55*A*, although widely different in size, regenerate orally with equal rapidity except in the final stages. Aborally the smaller piece regenerates more rapidly than the larger because its aboral end lies in a region of greater cellular activity. As regards the total amount of regeneration at both ends the small piece exceeds the laige piece, because of its position in the parent body. The only effect of size was noted at the final stage of the experiment when it was seen that the smaller pieces were falling behind the larger pieces with respect to size of the regenerated structures. In Series 35 the possible effect of size upon the final result was noted, but here also there was no visible effect during the earlier stages.

The results afforded by these two series are confirmed by a large number of experiments. In no case, when pieces were above a certain minimal size, could any effect of size upon rapidity of regeneration be observed during the earlier stages. In the later stages it was found that regeneration became less rapid and ceased in the smaller pieces earlier than in the larger. Even in this respect differences were slight in pieces of widely different size.

The amount of regeneration is then not proportional to the size of the piece. As regards both oral and aboral cut surfaces smaller pieces show a relatively much greater amount of regeneration than larger pieces ending at the same level. In a piece one tenth the length of the body the tentacles regenerate at first with the same rapidity as in a piece nine times as long. Only after the tentacles are well formed and several millimeters in length does a difference appear. In many pieces so short that they appeared to consist only of the regenerated disc and tentacles these organs were of the same size up to a late stage as in pieces many times as large.

As regards the nature of the effect of size in regeneration in *Cerianthus* and in many other forms a few points which have suggested themselves to me may be discussed in the hope of aiding in the analysis of this phase of the problem. It may be stated as a general rule that as regeneration advances the stimulus to regeneration diminishes. We may suppose that a certain amount of material or energy is necessary for the production of regenerated structures of a certain size. This energy is derived from the substance of the body in cases like the present where no food is supplied. The smaller pieces possess of course a smaller amount of material or energy available for regeneration. This is sufficient, however, for the earlier stages, but as the amount diminishes it is probably given up less readily by the other tissues. The well-known regulative power of animal tissues during starvation supports this view. Now as regeneration advances it is clear that in the smaller pieces the point where the stimulus to regeneration is no longer sufficient to cause the transfer of material or energy from the other tissues, would be reached earlier than in larger where the total supply of available material is much greater. Consequently regeneration in the smaller piece is retarded earlier than in the larger, even though the stimulus may be as great. The small piece is not necessarily exhausted, for if a new cut is made it may regenerate again, but the reserve supplies are held so closely by the other tissues that the decreasing regenerative stimulus is insufficient to render them available. A new cut surface means an increased stimulus, and under these conditions material which was not available during the later stages of the preceding regeneration may now be made available by the increased stimulus, though in this second regeneration the supply will run out sooner than before.

In the larger pieces of *Cerianthus* it is probable that the stimulus to regeneration ceases, or reaches the level of normal reparative stimuli, before a shortage of material occurs. A convenient designation for the condition of the smaller pieces is "relative exhaustion." They are not absolutely exhausted, since they may regenerate further under sufficiently powerful stimulus; they are, however, exhausted so far as the stimulus to which they are subjected is concerned. There is nothing new in the idea of relative exhaustion; it is a well-known phenomenon in biology and its rôle in regulative phenomena is undoubtedly important.

It has been impossible to determine with any degree of exactness the minimal size of pieces in which regeneration is possible. In small pieces the inrolling of the cut margins becomes so irregular that the piece often takes a form in which normal regeneration is impossible simply because of the relative position of parts. Moreover, since the regenerative power is different at different levels the minimal size of pieces differs according to the region of the body from which they are taken. Short pieces in the œsophageal region are not available for the aboral cut margin of the œsophagus unites with the aboral cut margin of the body-wall, thus producing a condition in which the œsophagus opens aborally as well as orally to the exterior and the enteron is completely closed. As will be shown at another time, regeneration in such pieces is always slight.

The smallest pieces in which normal regeneration has been observed were about one twentieth of the body-length and from the region just posterior to the œsophagus. When the regeneration was complete these pieces appeared to consist of little but tentacles and disc, which were only slightly smaller than in pieces many times as large. My observations prove that typical regeneration is possible in very small portions of the body, provided they represent complete transverse or oblique sections of the body; pieces in which the transverse continuity is interrupted roll up in such manner that typical regeneration can never occur.

The Effect of Temperature on Regeneration.

No special experiments in relation to temperature were performed, but my work upon *Cerianthus* extended from September, 1902, to February, 1903. In September the temperature of the water was very high; during October, as the weather became cooler, it fell gradually and continued to do so as long as observed, *i. e.*, until February. A comparison of similar series of pieces begun at different times during this period affords an interesting illustration of the effect of temperature. The various series for which data are given in this paper will serve for this purpose.]

Series 22 was begun September 24; Series 35, October 20; Series 45, November 7; Series 54, 55 and 56, December 15. If the series be considered in this order a considerable decrease in the rapidity of regeneration is noticeable. In Series 22, September 24, the marginal tentacles first appeared in the pieces *A* four days after section. In Series 54, 55, 56, December 15, the marginal tentacles in the pieces *A* from about the same level of the body as Series 22*A* appeared eleven days after section. Between these two extremes lie the other series, and in every case the later in the season the series was begun the less rapid the regeneration. It is not necessary to go over the data in detail to illustrate this point. In general it may be stated that the rapidity of regeneration in winter during the earlier stages is only about one third of the rapidity in summer.

The amount of regeneration as well as the rapidity also differs with the season. During September and October regenerated tentacles attained a length about twice as great as in similar pieces during January and February.

The length of time during which increase of size in regenerated structures, *e. g.*, tentacles, continues does not differ widely at high and low temperatures, and since regeneration is less rapid it is evident that the total amount must decrease with the temperature.

The most interesting point in connection with the effect of temperature on regeneration is the increase in size of the region at the aboral end in which regeneration does not occur. During the earlier months of my work the only pieces which failed to regenerate were the aboral tips comprising about one eighth of the body-length. Later, as illustrated in Series 45, an aboral piece about one fifth of the body-length failed to regenerate. In December, January and February pieces comprising more than the aboral third failed to regenerate or showed only slight traces of regeneration, as in Series 56*B*. Thus the portion of the body incapable of regeneration increases as temperature decreases.

In general, the effect of temperature upon regeneration in *Cerianthus* is what might be expected, since the activities of living substance in general increase and decrease with the temperature. At high temperatures it is only the extreme aboral end which cannot regenerate typically. From this "inactive" region regenerative power increases toward the oral end. As the temperature falls the limit of "inactivity" must advance toward the oral end, and more and more of the body be included in it.

The Number of Regenerated Tentacles.

It was determined that the number of tentacles regenerated is always less than the original number, and furthermore, that it decreases as the distance between the oral end of the piece and the oral end of the body increases.

It is difficult to determine with certainty the number of tentacles in living specimens of *C. solitarius*, but in fixed material they can readily be counted. A considerable amount of material fixed for this purpose was lost by accident when too late to replace it, so that exact data cannot be given at present. The decrease in the number of tentacles with increasing distance from the oral end is, however, always noticeable even on cursory examination of the pieces.

Another spieces was also employed for this purpose, viz: the small, whitish, undetermined species mentioned in the preceding paper. In this form the tentacles are much less numerous than in *C. solitarius*, and can usually be counted without difficulty in the living specimen. This species differs somewhat from *C. solitarius* in the extent of regenerative capacity, but the course of regeneration is the same, and since the structure is in general similar to that of the other members of the family, the results obtained from this species may be accepted as typical. In all cases the regenerated tentacles were counted only after they had attained a length of several millimeters; time being thus afforded for the establishment of the small tentacles in the growing region, which sometimes regenerate somewhat later than the others. Only marginal tentacles were counted since the labial tentacles are fewer in number than the intermesenterial chambers and regenerate less regularly than the marginal tentacles. The following series are given as examples:

Series 2, October 7. — Original number of marginal tentacles 21. After removal of disc and tentacles the body was cut into five pieecs, *A*, *B*, *C*, *D*, *E* (Fig. 8). Number of marginal tentacles regenerated *A*, 0, died; *B*, 15; *C*, 14; *D*, 13; *E*, 0, did not regenerate.

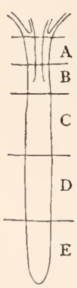

Fig. 8.

Series 5, October 19. — Original number of marginal tentacles 19. After removal of disc and tentacles body cut into five pieces, *A*, *B*, *C*, *D*, *E* (Fig. 9). Number of marginal tentacles regenerated: *A*, 0, died; *B*, 17; *C*, 15; *D*, 13; *E*, 0, did not regenerate.

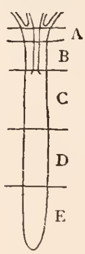

Fig. 9.

These two series are sufficient to show that the number of tentacles regenerated depends on position and has no relation to size of the piece. In both series the piece *B* is only half the size of *C*, yet it regenerates a larger number of tentacles.

These relations hold for all cases recorded, and, since the marginal tentacles correspond to the intermesenterial chambers, they are exactly what might be expected. The number of marginal tentacles which regenerate on a given piece is determined by the number of intermesenterial chambers which it possesses, or the number which it acquires by regeneration of mesenteries. The arrangement of mesenteries in *Cerianthus* was described in the preceding paper. All mesenteries appear first at the oral end and extend gradually in the aboral direction. In general, the younger a mesentery the less its length. Consequently as we pass aborally the number of mesenteries actually present decreases. After section at any level where regeneration is possible a powerful stimulus to growth exists; the mesenteries which extend into the piece remain and become united with the new œsophagus and others regenerate. It appears that the power to regenerate a given mesentery extends for a certain distance aboral to the end of that mesentery and since the mesenteries terminate at all levels we may now conclude that the "determination" of the body-wall for the regeneration of mesenteries shows a similar arrangement. Thus the youngest, shortest mesenteries can be regenerated only within a very short distance from the oral end, the next older and longer within a somewhat greater distance, and so on. The experimental results confirm and establish this conclusion in every case. The addition of new material to the body of *Cerianthus* in producing increase in circumference and number of tentacles and mesenteries may be conceived as a wedge of material which enters the body-wall at the oral end and continually forces its way aborally, thus separating the older parts of the body more and more widely. This conception of the method of growth in the transverse direction agrees with the view expressed above that the general reactive capacity of the tissues is greatest at the oral end. The stimulus to the formation of new mesenteries always becomes effective first at the oral end and from here gradually extends aborally until it is insufficient under ordinary conditions to give rise to a mesentery. Even at this level, however, a mesentery may be formed if the stimulus increases in intensity, or if the tissue becomes more susceptible to it. The resulting conditions give rise to rapid growth and we may suppose that formative stimuli which were insufficient to produce visible results so long as the piece formed an integral part of the parent-body, either become more powerful after artificial section or else that the new embryonic tissue becomes more susceptible.

A comparative account of the factors of position, size and temperature in *C. solitarius* and the other species studied is rendered unnecessary by the close agreement between all four species. Similar results were obtained in all cases. The only points worth noting here are slight differences in the degree of regenerative power. *Cerianthus membranaceus* regenerates less rapidly than the other species. In the small whitish undetermined species the aboral region in which typical regeneration does not occur is relatively much longer than in *C. solitarius* at the same temperatures. During October only about the aboral fifth or sixth of the body is incapable of regeneration or regenerates incompletely in *C. solitarius*, while in the other species a cut surface only a short distance aboral to the middle of the body is either incapable of regeneration or regenerates incompletely. This species is much less common than *C. solitarius* and was only rarely obtained; no opportunity offered of determining its regenerative power during the colder months. Presumably, however, regeneration would have failed to occur except in the region near the oral end.

One important result of this study may be summed up in the statement that the rapidity and the amount of regeneration in pieces from the body of *Cerianthus* depend primarily on the previous relations of pieces to the parent-body, *i. e.*, on the position and consequent functional condition. This fact is of importance as indicating that the phenomena of regeneration are due not to any special regulatory mechanism which bring about return to a "normal" or "typical" form, but to the same properties which cause growth and differentiation under normal conditions.

Summary.

1. The power of regeneration from cut surfaces in *Cerianthus* is greatest at the oral end and decreases aborally, becoming null a certain distance from the aboral end. This decrease of regen-

erative power is shown by differences both in rapidity and in amount of regeneration.

2. The size of the piece does not influence the rapidity or the amount of regeneration, except in the latest stages. Since the regenerative power is different at different levels, the minimal size of a piece capable of regeneration also differs at different levels, but inversely as the regenerative power.

3. Rapidity and amount of regeneration increase and decrease with the temperature.

4. The assumption of a special regulatory mechanism is not necessary for the explanation of form regulation in *Cerianthus*.

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